IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re application of | : | **Confirmation No. 1217** |
| Toshio SUZUKI et al. | : | Docket No. 2001_1875A |
| Serial No. 10/022,619 | : | Group Art Unit 1614 |
| Filed December 20, 2001 | : | Examiner Sandra E. Saucier |

PROCESS FOR PREPARATION OF (R)-1,2-PROPANEDIOL BY MICROBES

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## DEPOSIT DECLARATION

Assistant Commissioner for Patents,
Washington, D.C.

Sir:

Daiso Co., Ltd., Assignee of record of the above-identified application, by its undersigned duly authorized representative, hereby declares:

That microorganism Pseudomonas nitroreducens DS-S-RP8 has been deposited at the International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology of AIST Tsukuba Central 6, 1-1, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305-8566, Japan on June 29, 2001, under the Budapest Treaty, as Deposit No. FERM BP-7793;

That microorganism Alcaligenes sp. DS-S-7G has been deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, of 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305 Japan on November 15, 1989, under the Budapest Treaty, as Deposit No. FERM BP-3098;

That microorganism Pseudomonas sp. DS-SI-5 has been deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, of 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305 Japan on October 7, 1999, under the Budapest Treaty, as Deposit No. FERM BP-7080;

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That the undersigned is authorized to execute this agreement on behalf of the Assignee.

The undersigned declares further that all statements made herein of his own knowledge are true, and that all statements on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Daiso Co., Ltd.

Date: Feb. 28, 2003

Signature: Kouji SATOMO

Title: President

**Partial English translation of Japanese Patent Gazette**

(11) Japanese Patent Publication No. 6-30790

(43) Publication date: February 8, 1994

(71) Applicant Daicel Chem. Ind. Ltd.

(54) Title of Invention: A method for preparing an optically active 1,2-propandiol

**[Claim 1]** A method of preparing (R)-1,2-propanediol which is characterized in reacting an enantiomer mixture of 1,2-propandiol with a microorganism having an ability to make remain (R)-1,2-propanediol, or its treated product by reacting an enantiomer mixture of 1,2-prppandiol and then, isolating remaining (R)-1,2-propanediol.

**[Claim 2]** A method of preparing (S)-1,2-propanediol which is characterized in reacting an enantiomer mixture of 1,2-propandiol with a microorganism which belongs to a genus Pseudomonas and has an ability to make remain (S)-1,2-propanediol, or its treated product by reacting an enantiomer mixture of 1,2-propandiol and then, isolating remaining (S)-1,2-propanediol.

**[Claim 3]** The method of preparing (R)-1,2-propanediol according to claim 1, wherein the microorganism belongs to a genus Pseudomonas and has an ability to make remain (R)-1,2-propanediol by reacting an enantiomer mixture of 1,2-propandiol.

**[Claim 4]** A novel microorganism selected from the microorganisms which belong to a genus microorganism and an ability to metabolize and degrade either enantiomer of 1,2-propandiol.

**[0008]** In the present invention, the microorganisms used for preparing (R)-1,2-propanediol are not limited as long as the microorganisms have an ability to make remain (R)-1,2-propandiol by reacting an enantiomer mixture of 1,2-propandiol, but the microorganisms selected from a genus Pseudomonas are preferably used. The microorganisms used for preparing (S)-1,2-propanediol are not limited as long as the microorganisms belong to a genus Pseudomonas and have an ability to make remain (S)-1,2-propandiol by reacting an enantiomer mixture of 1,2-propandiol. Among the microorganisms belonging to a genus Pseudomonas having an ability to make remain (R)-compound are illustrated Pseudomonas putida TRB-2, TRP-4 and Pseudomonas sp. TRP-13. As a genus Pseudomonas having an ability to make remain (S)-compound are illustrated Pseudomonas putida TRP-7. These microorganisms include wild strains, mutants, or recombinant strains which are derived from gene technology or cell fusion technology and these all stains are preferably used. Furthermore, it is enough to use at least one strain.

**[0009]** Pseudomonas putida TRB-2, TRP-4 and Pseudomonas sp. TRP-13 and Pseudomonas putida TRP-7 were isolated from natural sources by the present inventors, which have a potent ability to metabolize or degrade stereoselectively either enantiomer of 1,2-propandiol. These strains were deposited at Japan Fermentation Research Institute Agency of Industrial Science and Technology, as Deposit No. FERM BP-3879, Deposit No. FERM BP-3880, Deposit No. FERM BP-3882, and Deposit No. FERM BP-3881, respectively.

The microbiological properties of each strain are shown in followings.

**[0012]** A medium for cultivating the microorganism used in the present invention is not limited as long as the microorganisms can grow therein. For example, as a carbon source, any one which the microorganisms mentioned above can utilize can be used. For example, carbohydrates such as glucose, fructose, sucrose or dextrin, alcohols such as sorbitol, glycerol, 1,2-propanediol or 1,3-propanediol, organic acids such as fumaric acid, citric acid, acetic acid, propionic acid or a salt thereof, hydrocarbons such as paraffin and a mixture thereof can be used. As a nitrogen source, ammonium inorganic acid salt, such as ammonium chloride, ammonium sulfate or ammonium phosphate, ammonium organic acid salts, such as ammonium fumarate or ammonium citrate, compounds containing an organic or

inorganic nitrogen, such as meat extract, yeast extract, coan steep liquor, hydrolyzed casein, urea, or a mixture of them, can be used. Further nutritious sources ordinally used in the culture medium, such as inorganic salts, a small amount of metal salts or vitamines can be used. Furthermore, a factor which promotes the growth of the microorganism, or a factor which promotes an ability of production of the objective compound of the present invention, or $CaCO_3$ which is effective to maintain pH value in the culture medium can be, if necessary added.

**[0013]** The cultivation is carried out, in the medium of which pH is 3.0-10.0, preferably 4-8, at temperature 20-45°C, preferably 25-37°C, aerobically or anaerobically under a condition suitable for growth of the microorganism for 5-120 hours, preferably about 12-72 hours.

**[0014]** The methods for preparing an optically active 1,2-propanediol from an enantiomer mixture of 1,2-propanediol include a method for making reaction by adding an enantiomer mixture of 1,2-propanediol to a culture broth itself, or to a suspension prepared by re-suspending the cells in a buffer solution or water after separating the cells by centrifugation, or after washing the separated cells. In this reaction, it may be preferable to add a carbon source such as glucose or sucrose as an energy source. The cells may be intact, and disrupted cell lysate,

acetone-treated cells or freezed dried cells can be used, too. Further, the cell or its treated product which is immobilized by a known method such as polyacrylamide gel method, sulfur-containing polysaccharide method (carrageenan gel method), argininic acid gel method, or agar gel method can be used. Furthermore, enzymes extracted and purified from the cell-treated product by a method combined by known methods can be used.

**[0015]** An enantiomer mixture of 1,2-propanediol itself, a solution dissolved it in water or in an inert solvent or a dispersion of it in a detergent may be once or in parts added . The reaction is carried out at pH 3-10, preferably pH 5-9, at 10-60°C, preferably 20-40°C, for about 1 to 120 hours, under stirring or standing. The concentration of an enantiomer mixture of 1,2-propanediol, a substrate is not limited, but about 1-40% in the concentration is preferable. When pH in the reaction mixture is maintained using NaOH, $CaCO_3$, HCl, $H_2SO_4$ and so on, the better result may be obtained.

**[0016]** The optically active 1,2-propanediol remained by the reaction is easily isolated from the reaction medium directly, or is easily isolated by using a conventional purification method such as extraction with an solvent, distillation, or column chromatography after separation of the cells. When aldehyde or keton is produced as a

reaction by-product, it is also effective to exclude it by treating with a sodium sulfite solution.

**[0017]**

**[Example]** The present invention is explained by the following examples, but the present invention is not limited by only these examples.

"%" in the examples means % by weight except for that specially mentioned.

The quantitative analysis of 1,2-propanediol is easily conducted by using gas chromatography (Column: Thermon 3000 5%/chromosorb W 80-100 mesh (Φ 3mm x 2.1m), at 140°C).

The measurement of the optical purity was conducted as follows: An optically active 1,2-propanediol prepared by this reaction was reacted with phenylisocyanate in a conventional way to prepare a carbamoyled compound thereof. The carbamoyled compound was measured by HPLC using a column for optical resolution (Column: Chiralcell OD manufactured by Daisel Chem. Ind. Ltd., Solvent: n-hexane/2-propanol=16.3, Wave length 254nm, Flow rate 1.0ml/min., at 45°C, Retention time (R)-form: 14.2min., (S)-form: 23.5min.)

**[0018]** Example 1

The following culture medium (50ml) for preparing cells was poured in a 500 ml Sakaguti-flask. After being sterilized each microorganism shown in Table 1 was inoculated and the

medium was shaken at 30°C for 50 hours. Then the cells were collected by centrifugation to get intact cells.

<Culture medium for preparing cells>

| | |
|---|---|
| Racemic 1,2-propanediol | 1.0% |
| Meat extract | 0.1% |
| Yeast extract | 0.1% |
| Polypeptone | 0.2% |
| $KH_2PO_4$ | 0.07% |
| $(NH_4)_2HPO_4$ | 0.13% |
| $MgSO_4.7H_2O$ | 0.05% |
| pH 7.2 | |

The intact cells were suspended in deionized water to prepare a total volume of 25ml. Each 12.5ml thereof was poured in two 500ml Sakaguti-flasks (A, B). A mixture (12.5ml) of 12% racemic 1,2-propanediol/6% $CaCO_3$ was poured in flask A, and a mixture (8.34ml) of 12% racemic 1,2-propanediol/6% $CaCO_3$ was poured in flask B and further in the flask B deionized water (4.16ml) was poured. The both mixtures were reacted under shaking at 30°C for 48 hours. (Final concentration: flask A: 6% 1,2-propanediol, 3% $CaCO_3$; flask B: 4% 1,2-propanediol, 2% $CaCO_3$)

**[0019]**

After the reaction, the cells were removed by centrifugation. The supernatant (2ml) obtained was

saturated with sodium chloride and the remaining 1,2-propanediol therein was extracted with ethyl acetate (2ml). The solvent was removed from the extract and to the residue was added phenylisocyanate (50µl) to make phenylcarbamoylation. To this solution was added HPLC mobile phase (2ml) (n-hexane/2-propanol 16:3) to dissolve it. The optical purity was measured by HPLC. The above supernatant was diluted with water, if necessary and the amount of 1,2-propanediol was measured by gas chromatography. The obtained absolute configuration and optical purity and residual amount of 1,2-propanediol were shown in Table 1.

**[0020]**

**[Table 1]**

| Microorganism | Initial conc. of 1,2-propandiol(%) | Residual amount of 1,2-propanediol (g/L) | Absolute Conf. | Optical purity of 1,2-propandiol(%ee) |
|---|---|---|---|---|
| Psudomonas putida TRB-2 | 4 | 19.1 | R | >99 |
| | 6 | 32.8 | R | 78.9 |
| Pseudomonas putida TRP-4 | 4 | 16.6 | R | >99 |
| | 6 | 30.2 | R | 91.5 |
| Pseudomonas sp. TRP-13 | 4 | 9.7 | R | >99 |
| | 6 | 26.3 | R | >99 |
| Pseudomonas putida TRP-7 | 4 | 17.6 | S | >99 |
| | 6 | 26.5 | S | >99 |

**[0021]** Example 2

Pseudomonas sp. TRP-13 and Pseudomonas putida TRP-7,

according to the method of Example 1, were cultivated in a culture medium (50ml) poured in a 500 ml Sakaguti-flask for 50 hours, respectively. After centrifugation the collected cells were suspended in deionized water to prepare a total volume of 25ml. Each 12.5ml thereof was poured in two 500ml Sakaguti-flasks (A, B) in the same manner as in Example 1. A mixture (10ml) of 20% racemic 1,2-propanediol/10% $CaCO_3$ and deionized water (2.5ml) were added to flask A (Final concentration: 8% 1,2-propanediol, 4% $CaCO_3$), and a mixture (12.5ml) of 20% racemic 1,2-propanediol/10% $CaCO_3$ were added to flask B (Final concentration: 10% 1,2-propanediol, 5% $CaCO_3$). The both mixtures were reacted under shaking at 30°C for 72 hours. After the reaction, residual amount, optical purity and absolute configuration of 1,2-propanediol were measured in the same manner as in Example 1. The results were shown in Table 2.

**[0022]**

**[Table 2]**

| Microorganism | Initial conc. of 1,2-propandiol(%) | Residual amount of 1,2-propanediol (g/L) | Absolute Conf. | Optical purity of 1,2-propandiol(%ee) |
|---|---|---|---|---|
| Pseudomonas sp. TRP-13 | 8 | 42.8 | R | 96.8 |
| | 10 | 63.7 | R | 65.1 |
| Pseudomonas putida TRP-7 | 8 | 37.6 | S | >99 |
| | 10 | 47.8 | S | >99 |

[0023] Example 3

The same culture medium (1.2L) as in Example 1 was poured in a small fermenter (2L) manufactured by B. Blawn Ltd. After being sterilized at 121°C for 15 min. and being cooled, 12ml of the culture broth in which Pseudomonas sp. TRP-13 was cultivated under shaking in the medium shown below at 30°C for 25 hours (10ml/Ø21mm) were aseptically inoculated and the medium was cultivated at 30°C, 700rpm, 0.5vvm, for 18.5 hours.

<Culture medium>

| | |
|---|---|
| Glucose | 0.5% |
| Meat extract | 0.3% |
| Yeast extract | 0.3% |
| Polypeptone | 0.5% |
| $KH_2PO_4$ | 0.07% |
| $(NH_4)_2HPO_4$ | 0.13% |
| $MgSO_4.7H_2O$ | 0.05% |
| pH 7.2 | |

After cultivation, the culture broth (1L) was centrifuged and the obtained intact cells were suspended in deionized water to prepare a total volume of 200ml. A mixture (800ml) of 7.5% racemic 1,2-propanediol/3.75% $CaCO_3$ was added thereto (Final concentration: 6% 1,2-propanediol/3% $CaCO_3$) and the mixture was reacted in the above furmenter

at 30°C, 700rpm, 0.5vvm, for 79.5 hours.

The optical purity of (R)-1,2-propanediol was 99.8% and the yield of (R)-1,2-propanediol was 97.7% when the reaction was completed.

**[0024]**

The reaction mixture was subject to centrifugation to give supernatant. The supernatant was filtered by ultrafilter (M.W.10000 cut) to remove higher molecular weight compounds. The filtrate was concentrated to 106g with a rotary evaporator. The resulting white crystals were removed by filtration. The filtrate was again concentrated to 40.2g. The condensed solution was extracted with ethyl acetate. The ethyl acetate layers were combined and the solvent was removed from the extract. To the residue was added a saturated aqueous solution of sodium hydrogen sulfite (5ml) and the mixture was stirred at room temperature for 1 hour. Diethy ether was added thereto and the ether layer was dried on anhydrous sodium sulfate and the solvent was removed. The residue was distilled at a pressure of 5mm Hg to give purified (R)-1,2-propandiol (8.6g). The area ratio of gas chromatography: 98.4%, optical purity: 99.3%e.e., $[\alpha]^{25}_{D}$ -23.8° (c=0.992, $H_2O$)

**Partial English translation of Japanese Patent Gazette**

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(71) Applicant: Daicel Chem. Ind. Ltd.

(54) Title of invention: A method for preparing an optically active alcohol

**[Claim]**

**[Claim 1]** A method for preparing an optically active alcohol having a configuration represented by the following structure (III) or (IV), which is characterized in reacting an enantiomer mixture of an alcohol represented by the following formula (I) or (II) with a microorganism which belongs to a genus Pseudomonas and has an ability to make remain an optically active alcohol having a configuration represented by the following structure (III) or (IV) by reacting an enantiomer mixture of an alcohol represented by the following formula (I) or (II), or its treated product, and then isolating a remaining optically active alcohol having a configuration represented by the following structure (III) or (IV).

( I ) ( II )

in which R is H, $NH_2$, Cl, Br, I, or $-CH_2OH$, m is an integer

1-5, and n is an integer 0-3.

( III ) ( IV )

in which R, m and n are the same as defined above.

**[0015]** Alcohols represented by the formula (I) used in the present invention include diols, e.g. 1,2-butanediol, 1,2-pentanediol or 1,2-hexanediol, triols, e.g. 1,2,4-butanetriol, 1,2,5-pentanetriol or 1,2,6-hexanetriol, halogenoalcohols, e.g. 3-chloro-1,2-propanediol or 3-buromo-1,2-propanediol, and aminoalcohls, e.g. 3-amino-1,2-propanediol. Alcohols represented by the formula (II) include 2-amino-1-propanol, 2-amino-1-butanol and 2-amino-1-pentanol.

**[0016]** The microorganisms used in the present invention are not limited as long as the microorganisms belong to a genus Pseudomonas and have an ability to make remain an optically active alcohol represented by the structure (III) or (IV) by reacting an enantiomer mixture of an alcohol represented by the formula (I) or (II). Pseudomonas putida TRB-2, TRP-4 and Pseudomonas sp. TRP-13 are illustrated.

These microorganisms include wild strain, mutants, or recombinant strains which are derived from gene technology

or cell fusion technology and these all stains are preferably used. Furthermore, it is enough to use at least one strain.

**[0017]** Pseudomonas putida TRB-2, TRP-4 and Pseudomonas sp. TRP-13 were isolated from natural sources by the present inventors, which have a potent ability to metabolize or degrade stereoselectively either enantiomer of an alcohol represented by the formula (I) or (II). These strains were deposited on July 3, 1992 at Japan Fermentation Research Institute Agency of Industrial Science and Technology as Deposit No. FERM BP-3879, Deposit No. FERM BP-3880 and Deposit No. FERM BP-3882, respectively.

The microbiological properties of each strains are shown in followings.

**[0019]** The medium for cultivation of microorganisms used in the present invention is not limited as long as the microorganism can grow therein. For example, a carbon source is not limited as long as the microorganism can grow, but includes carbohydrates such as glucose, fructose, sucrose or dextrin, alcohols such as sorbitol, glycerol, 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 2-amino-1-propanediol or 2-amino-1-butanol, organic acids such as fumaric acid, citric acid, acetic acid, propionic acid or a salt thereof, hydrocarbons such as paraffin and a mixture thereof. As a nitrogen source, ammonium inorganic acid

salt, such as ammonium chloride, ammonium sulfate or ammonium phosphate, ammonium organic acid salts, such as ammonium fumarate or ammonium citrate, compounds containing an organic or inorganic nitrogen, such as meat extract, yeast extract, coan steep liquor, hydrolyzed casein, urea, or a mixture of them, can be used. Further nutritious sources ordinally used in the culture medium, such as inorganic salts, a small amount of metal salts or vitamines can be used. Furthermore, a factor which promotes the growth of the microorganism, or a factor which promotes an ability of production of the objective compound of the present invention, or $CaCO_3$ which is effective to maintain pH value in the culture medium can be, if necessary added.

**[0020]** The cultivation is carried out, in the medium of which pH is 3.0-10.0, preferably 4-8, at 20-45°C, preferably25-37°C, aerobically or anaerobically under a condition suitable for growth of the microorganism for 5-120 hours, preferably 12-72 hours.

**[0021]** The methods for preparing an optically active alcohol represented by the formula (III) or (IV) starting from an enantiomer mixture of alcohols represented by the formula (I) or (II) include a method for making reaction by adding an enantiomer mixture of alcohols represented by the formula (I) or (II) to a culture broth itself, or to a suspension prepared by suspending the cells in a buffer

solution or water after separating the cells by centrifugation or washing the cells. In this reaction, it may be preferable to add a carbon source such as glucose or sucrose as an energy source. Cells may be intact, and disrupted cell lysate, acetone-treated cells or freezed dried cells can be used, too. Further, the cell or its treated product which is immobilized by a known method such as polyacryl gel method, sulfur-containing polysaccharide method (carrageenan gel method), argininic acid gel method, or agar gel method can be used. Furthermore, enzymes extracted and purified from a cell-treated product by a method combined by known methods can be used.

**[0022]** An enantiomer mixture of alcohols represented by the formula (I) or (II), itself, a solution dissolved it in water or in an inert solvent or a dispersion of it in a detergent may be once or in parts added. The reaction is carried out at pH 3-10, preferably pH 5-9, at 10-60°C, preferably 20-40°C, for about 1 to 120 hours, under stirring or standing. The concentration of an enantiomer mixture of alcolhols represented by the formula (I) or (II), a substrate is not limited, but about 1-40% in the concentration is preferable. When pH in the reaction medium is maintained using NaOH, $CaCO_3$, HCl, $H_2SO_4$ and so on, the better result may be obtained.

**[0023]** The optically active of alcohols represented by the

formula (III) or (IV) remained by the reaction is easily isolated from the reaction mixture directly, or is easily isolated by using a conventional purification method such as extraction with an solvent, distillation, or column chromatography after separation of the cells. When aldehyde or ketol as a reaction by-product is produced, it is also effective to exclude it by treating with sodium sulfite solution.

**[0024]**

**[Example]** The present invention is explained by the following examples, but the present invention is not limited by only these examples.

"%" in the examples means % by weight except for that specially mentioned.

**[0025]** Examples 1-8

The following culture medium (50ml) for preparing cells was poured in a 500 ml Sakaguti-flask and the medium was sterilled at 121°C for 15 min. After being cooled, a liquid preculture medium 0.5ml of Pseudomonas sp. TRP-13 (cultivated at 30°C for 24 hours in the following preculture medium by using a test tube 5ml/Φ 21mm) was inoculated thereto. After cultivation with shaking at 30°C for 48 hours, the intact cells were collected by centrifugation. The cells were suspended in deionized

water to prepare a total volume of 25ml. A solution 500ml containing each racemic alcohol shown in Table 1 was prepared, respectively (The solution of aminoalcohol is adjusted to pH 7 with NaOH.). A cell-suspension 2.5ml, a substrate solution 2.5ml and $CaCO_3$ 0.05g were mixed and the mixture was reacted under shaking in a test tube (Φ 21mm) at 30°C for 12-24 hours. After reaction, the cells were removed by centrifugation to give the supernatant. An optical purity of each alcohol and a yield of each enantiomer in the supernatant obtained thus were measured under the conditions shown in Table 2 and Table 3, respectively. The measurement of the optical purity was conducted by HPLC using a column (Φ 4.6 x 250mm): Daicel Chem. Ind. Ltd.

**[0026]** < Preculture medium >

| | |
|---|---|
| Glucose | 0.5% |
| Meat extract | 0.3% |
| Yeast extract | 0.3% |
| Polypeptone | 0.5% |
| $KH_2PO_4$ | 0.07% |
| $(NH_4)_2HPO_4$ | 0.13% |
| $MgSO_4.7H_2O$ | 0.05% |
| pH 7.2 | |

<Culture medium for preparing cells>

| | |
|---|---|
| Racemic 1,2-propanediol | 1.0% |
| Meat extract | 0.1% |
| Yeast extract | 0.1% |
| Polypeptone | 0.2% |
| $KH_2PO_4$ | 0.07% |
| $(NH_4)_2HPO_4$ | 0.13% |
| $MgSO_4.7H_2O$ | 0.05% |
| pH 7.2 | |

**[0027]**

**[Table 1]**

TRP-13 strain Substrate 250mM $CaCO_3$ 1%

| Example | Substrate | Reaction time(hr) | Residual substrate | | | |
|---|---|---|---|---|---|---|
| | | | Conc. (mM) | Yield* (%) | Configuration | Optical purity (% ee) |
| 1 | 1,2-Butanediol | 24 | 77 | 60 | R | 95.4 |
| 2 | 1,2-Pentanediol | 24 | 147 | 78 | R | 32.1 |
| 3 | 1,2,4-Butanetriol | 12 | 67 | 54 | R | >99.0 |
| 4 | 1,2,6-Hexanetriol | 12 | 116 | 93 | R | >99.0 |
| 5 | 3-Cloro-1,2-propanediol | 24 | 106 | 85 | S | >99.0 |
| 6 | 2-Amino-1-propanol | 24 | 148 | 90 | R | 51.2 |
| 7 | 2-Amino-1-butanol | 24 | 206 | 100 | R | 24.9 |
| 8 | 3-Amino-1,2-propanediol | 24 | 188 | 100 | R | 43.5 |

**[0031]**

Example 9-16

In the same manner as in Example 1-8 by using Pseudomonas

putida TRB-2 in stead of Pseudomonas sp. TRP-13, there were obtained the results shown in following Table 4. Remaining optically active alcohols were likewise measured.

**[0032]**

**[Table 4]**

TRB-2 strain Substrate 250mM $CaCO_3$ 1%

| Example | Substrate | Reaction time(hr) | Residual substrate | | | |
|---|---|---|---|---|---|---|
| | | | Conc. (mM) | Yield* (%) | Configuration | Optical purity (% ee) |
| 9 | 1,2-Butanediol | 24 | 77 | 60 | R | 95.4 |
| 10 | 1,2-Pentanediol | 24 | 202 | 100 | R | 23.8 |
| 11 | 1,2,4-Butanetriol | 12 | 53 | 42 | R | >99.0 |
| 12 | 1,2,6-Hexanetriol | 24 | 108 | 87 | R | >99.0 |
| 13 | 3-Cloro-1,2-propanediol | 24 | 144 | 98 | S | 70.1 |
| 14 | 2-Amino-1-propanol | 24 | 119 | 95 | R | >99.0 |
| 15 | 2-Amino-1-butanol | 24 | 124 | 93 | R | 88.0 |
| 16 | 3-Amino-1,2-propanediol | 24 | 154 | 100 | R | 82.7 |

**[0033]** Example 17-24

In the same manner as in Example 1-8 by using Pseudomonas putida TRB-4 in stead of Pseudomonas sp. TRP-13, there were obtained the results shown in following Table 5. Remaining optically active alcohols were likewise measured.

**[0034]**

**[Table 5]**

TRP-4 strain Substrate 250mM $CaCO_3$ 1%

| Example | Substrate | Reaction time(hr) | Residual substrate | | | |
|---|---|---|---|---|---|---|
| | | | Conc. (mM) | Yield* (%) | Configuration | Optical purity (% ee) |
| 17 | 1,2-Butanediol | 24 | 88 | 68 | R | 92.7 |
| 18 | 1,2-Pentanediol | 24 | 194 | 85 | R | 9.3 |
| 19 | 1,2,4-Butanetriol | 12 | 63 | 51 | R | >99.0 |
| 20 | 1,2,6-Hexanetriol | 24 | 97 | 73 | R | 89.6 |
| 21 | 3-Cloro-1,2-propanediol | 24 | 112 | 85 | S | 90.2 |
| 22 | 2-Amino-1-propanol | 24 | 154 | 97 | R | 56.8 |
| 23 | 2-Amino-1-butanol | 24 | 213 | 97 | R | 14.6 |
| 24 | 3-Amino-1,2-propanediol | 24 | 169 | 99 | R | 47.2 |